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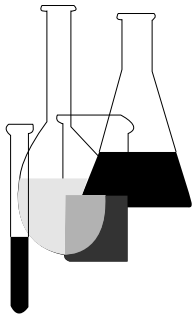
Ecological Effects Test Guidelines

OPPTS 850.2300
Avian Reproduction Test



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 850.2300 Avian reproduction test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 797.2130 Bobwhite Reproduction Test and 797.2150 Mallard Reproduction Test; OPP 71–4 Avian Reproduction Test (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982; and OECD 206, Avian Reproduction Test.

(b) **Purpose.** This guideline is designed to develop data on the reproductive effects on the bobwhite and mallard of chemical substances and mixtures subject to chronic environmental effects test regulations. The Agency will use these and other data to assess the reproductive effects on birds that these chemicals may present to the environment. The prerequisite data for the study are water solubility, vapor pressure, and avian dietary LC50 of the test substance. Guidance information on the substance include the structural formula, purity, *n*-octanol/water partition coefficient, methods for quantification of the test substance in the diet, and its stability in water, light, and in the diet.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline.

(1) In addition, the following definitions apply generally to this guideline:

Acclimation is physiological and behavioral adaptation to environmental conditions (e.g. housing and diet) associated with the test procedure.

Basal diet is the untreated form of the diet, such as the diet obtained from a commercial source.

Photoperiod is the light/dark periods in a 24 h day. This is usually expressed in a form such as 17 h light/7 h dark or 17L/7D.

Test substance is the specific form of a chemical or mixture of chemicals that is used to develop the data.

(2) The following definitions refer specifically to the production and quality of eggs and the subsequent development of these eggs up to the point where young are 14 days old.

Eggs cracked. Eggs determined to have cracked shells when inspected with a candling lamp. Fine cracks cannot be detected without using a can-

dling lamp and if undetected will bias data by adversely affecting embryo development. Values are expressed both as number of eggs per pen per test and as a percentage of eggs laid by all hens during the test.

Eggs laid. This term refers to the total egg production during the test, which normally includes 10 weeks of laying. Values are expressed as numbers of eggs per pen per season (or test).

Eggs set. All eggs placed under incubation, i.e. total eggs minus cracked eggs and those selected for analysis of eggshell thickness. The number of eggs set, itself, is an artificial number, but it is essential for the statistical analysis of other development parameters.

Eggshell thickness. The thickness of the shell and the membrane of the egg at several points around the girth after the egg has been opened, washed out, and the shell and membrane dried for at least 48 h at room temperature. Values are expressed as the average thickness of the several measured points in millimeters.

14–day–old survivors. Birds that survive for 2 weeks following hatch. Values are expressed both as a percentage of hatched eggs and as the number per pen per season (test).

Hatchability. Embryos that mature, pip the shell, and liberate themselves from the eggs on day 23 or 24 of incubation for bobwhite and days 25, 26, or 27 of incubation for mallards. Values are expressed both as number of hatchlings per pen per test and as percentage of viable embryos (fertile eggs).

Live 18–day embryos or 21–day embryso for bobwhite and mallards, respectively. Embryos that are developing normally after 18 or 21 days of incubation for bobwhite and mallards. This is determined by candling the eggs. Values are expressed both as number of 18–day old quail or 21–day old duckling embryos per pen per test and as a percentage of viable embryos (fertile eggs).

Viable embryos (fertility). Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 11 days after incubation has begun. It is difficult to distinguish between the absence of fertilization and early embryonic death. The distinction can be made by breaking out eggs that appear infertile and examining further. This distinction is especially important when a test compound induces early embryo mortality. Values are expressed as a percentage of eggs set.

(d) **Test procedures**—(1) **Summary of the test.** (i) The birds should be observed for health and acclimated for at least 2 weeks after arrival.

(ii) Test birds should be randomly assigned to control and various treatment groups.

(iii) The test substance should be thoroughly and evenly mixed into the diet. All treatment levels should be analyzed for test substance concentrations at the beginning and midway through the test.

(iv) Birds should be weighed at the beginning of the test, at 14–day intervals until the onset of laying, and at termination of the test.

(v) Photoperiod should be carefully controlled on a shortday basis during the initial exposure phase, then increased to 16 to 17 h to induce egg laying.

(vi) Birds should be observed regularly for abnormal behavior or mortality throughout the test.

(vii) Eggs should be removed daily and stored until there is a sufficient quantity for incubation. All eggs should be candled at day 0 for cracks and cracked eggs removed. Once every 2 weeks, all eggs produced that day should be analyzed for eggshell thickness. Incubated eggs should be candled on day 11 and day 18 for bobwhite and day 14 and day 21 for mallards. Hatching should be completed by day 24.

(viii) Hatchlings should be maintained until they are 14 days old. Abnormal behavior or death should be reported. Hatchlings should be weighed on day 14.

(ix) A statistical analysis should be performed, preferably by analysis of variance or regression analysis.

(x) The report should include all conditions, procedures, and results. Data should be sufficiently detailed for an independent statistical analysis.

(xi) All treated birds should be sacrificed and disposed of properly. Control birds may be kept as breeding stock, but should not be used in any other tests. Control offspring may be reared and used in another test as adults.

(2) **Definitive test**—(i) **Test substance.** (A) Concentrations for the test substance should be based on measured or calculated residues expected in the diet, unless otherwise specified. The concentrations should include an actual or expected field residue exposure level and a multiple level such as 5. Generally, three treatment groups and a control group will be used. The highest nonlethal level may be estimated from the avian dietary LC50.

(B) The material to be tested should be analytically pure and the degree of purity should be reported along with the percentage of each impurity. The technical grade should be tested.

(ii) **Controls.** A concurrent control is required during every test. The control birds should be from the same hatch as the test groups. Control

and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be added to the diets of control birds. If a carrier or diluent is used in preparation of the test diets, the same carrier should be added to the diets of control birds in the highest concentration used for test diets.

(iii) **Test groups and numbers of birds.** (A) For bobwhite, each of the three treatment groups and the control group should consist of a minimum of 12 replicate pens. Each pen should contain one male and one female, or alternatively one male and two females. For mallards each of the three treatment groups and the control group shall consist of a minimum of 12 replicate pens, with each pen containing one male and one female or with each pen containing one male and three females and with a minimum of 8 replicates. The use of 20 replicate pens in the control group may yield a test with greater statistical power. Either arrangement is acceptable if productivity reaches the definitive values given in paragraph (d)(2)(xi)(A) of this guideline. Because the behavioral interactions of birds in the two arrangements are likely to be different, testing facilities using an arrangement with which they are not familiar are advised to experiment first without test substances in order to determine the feasibility of obtaining acceptable productivity levels.

(B) All control and treatment birds should be randomly distributed to pens from the same population.

(iv) **Duration of test.** (A) The test consists of three phases following acclimation to test facilities. The initial phase begins with exposure of treatment groups to diets containing the test substance and is typically 6 to 8 weeks long. After the initial phase, the photoperiod is manipulated according to paragraph (d)(4)(v) of this guideline to bring the hens into laying condition. This second phase ends with the onset of egg-laying and is typically 2 to 4 weeks long. The final phase begins with the onset of laying and lasts for at least 8 weeks, preferably 10 weeks. A withdrawal study period may be added to the test phase if reduced reproduction is observed. The withdrawal period, if used, need not exceed 3 weeks.

(B) Exposure of adult birds to the test substance should be continuous throughout the test. Unless otherwise specified, test birds should be exposed for at least 10 weeks prior to the onset of egg laying.

(v) **Preparation for reproduction (photoperiod).** (A) Lighting regimes (photoperiod) are critical to successful reproduction. Various photoperiod regimes have been demonstrated to give acceptable results. Any photoperiod regime that results in productivity that meets the definitive values given in paragraph (d)(4)(xii) of this guideline is acceptable as long as birds are exposed to treated diets a minimum of 10 weeks prior to the onset of laying. Regardless of the methods selected, lighting should

be controlled carefully. It is important during the initial phase to not interrupt the dark period unless absolutely necessary.

(B) A suggested photoperiod regime would consist of maintaining birds under a photoperiod for 7 or 8 h of light during the initial phase. At the end of the initial phase, the photoperiod may be increased to 16 to 17 h of light per day. The photoperiod may be maintained at this level for the remainder of the study, although an increase each week of 15 min/day is acceptable.

(vi) **Observations of record on adult birds.** (A) Body weights should be recorded for each adult bird at the beginning of the treatment period, at 14–day intervals until the onset of egg laying, and at termination of treatment. Taking of body weights during egg laying is discouraged because of possible adverse effects on egg production. Food consumption should be measured and recorded by pen as often as body weights are measured prior to the onset of laying and at least biweekly throughout the rest of the study.

(B) Observations on adult birds should be made at least once a day. Any mortality or other signs of toxicity should be described and recorded by date or day of test. Gross pathological examinations should be conducted on all birds that die during the test period, and for all survivors at the end of the test. Analysis of two or more tissues (e.g. muscle, fat) for test substance residues is encouraged, but not required unless the test substance is persistent, bioaccumulates, or if the log P (*n*-octanol/water) is higher than 3.

(vii) **Egg collection, storage, and incubation.** All eggs should be collected daily, marked according to the pen from which collected, and should be stored at 16 °C and 55 to 80 percent relative humidity. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. At weekly or biweekly intervals, eggs should be removed from storage and be candled to detect eggshell cracks. Except for eggs with cracked shells and those eggs removed for eggshell thickness measurements, all eggs should be set after candling for incubation in a commercial incubator. If incubators are not equipped to turn eggs automatically, they should be turned daily by hand. During the incubation period, eggs should be maintained at 37.5 °C and approximately 70 percent relative humidity. Eggs should be candled again on day 11 of incubation to determine fertility and early death of embryos. A final candling should be done on day 18 to measure embryo survival. On day 21, eggs should be removed to a separate incubator or hatcher. Hatching will normally be complete by the end of day 24. During hatching the temperature and relative humidity should be 37.5 °C and 70 percent, respectively. Forced draft incubators and hatchers should be used. If still-air, gravity-vented incubators and hatchers are used, temperatures should be 1.5–2 °C higher, and the relative humidity should be increased by about 10 percent. At higher ele-

vations, higher relative humidity is necessary. Temperatures in brooder pens should be measured at 2.5 to 4 cm above the pen floor.

(viii) **Chick or duckling maintenance.** By day 24 or 27 of incubation, the hatched bobwhite chicks and ducklings, respectively, should be removed from the hatcher or incubator. Chicks or ducklings should be either housed according to the appropriate parental pen group or individually marked (such as by leg bands) as to parental group and housed together. Chicks or ducklings should be maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal or stainless steel. Temperature in the pens should be controlled, preferably by a thermostatically controlled device. A temperature gradient in the pen from approximately 35 °C to approximately 22 °C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Chicks or ducklings should be provided a standard commercial game bird starter ration, or its nutritional equivalent. No test substance may be added to the diet of chicks or ducklings. Chicks or ducklings should be maintained until they are 14 days old. Lighting should be on a diurnal basis (e.g. 14 h of light, 10 h of dark, with a 15–30 min transition at dawn and dusk, but other lighting regimes are acceptable.

(ix) **Observations of record on chicks or ducklings.** The number of embryos that mature, embryos that pip shell, and embryos that liberate themselves, hatchability, percentage of normal hatchlings, percentage of 14–day old survivors, and number of 14–day old survivors per hen should be recorded and reported. Chicks or ducklings should be observed daily from hatching until they are 14 days old. Mortality, signs of toxicity, and other clinical abnormalities should be recorded at least cumulatively through day 5 and recorded by age from days 5 through 14. Average body weights should be recorded for chicks or ducklings at day 14.

(x) **Eggshell thickness.** Once every 2 weeks all eggs newly laid that day should be removed and measured for eggshell thickness. Eggs should be opened at the girth (the widest portion), the contents washed out (or used or saved for egg residue analysis), and the shell air-dried for at least 48 h. The thickness of the shell plus the dried membrane should be measured at a minimum of 3 points around the girth using a micrometer calibrated at least to 0.01 mm units.

(xi) **Typical observed values.** The values reported here represent those observed from a few testing facilities under their conditions. These values are not necessarily representative of those from all facilities, but if a reproduction test does not meet or at least approach these values for control birds, then there is likely to be a problem with test procedures or conditions that should be investigated and corrected.

(A) **Eggs laid.** Normal values for both bobwhite and mallards—28 to 38 eggs per hen per season.

(B) **Eggs cracked.** Normal values for bobwhite—0.6 to 2.0 percent of eggs laid. Normal values for mallards—0.6 to 6 percent of eggs laid.

(C) **Viable embryos (fertility).** Normal fertility values for bobwhite—75 to 90 percent of eggs set. Normal fertility value for mallards—85 to 98 percent of eggs set.

(D) **Live 18–day embryos.** Normal values for bobwhite and mallards—97 to 99 percent of viable embryos.

(E) **Hatchability.** Normal values for bobwhite and mallards—50 to 90 percent of viable embryos (fertile eggs).

(F) **14–day–old survivors.** Normal values for bobwhite—75 to 90 percent of eggs hatched. Normal value for mallards—94 to 99 percent of eggs hatched.

(G) **Eggshell thickness.** Normal average values for bobwhite—0.19 to 0.24 mm. Normal values for mallards—0.34 to 0.39 mm.

(xii) **Definitive test criteria.** (A) A test is unacceptable if bobwhite chick or mallard duckling productivity in control groups does not average 12 or 10, respectively, 14-day old survivors per pen over a 10–week period.

(B) A test is unacceptable if the average eggshell thickness in control groups is less than 0.19 mm for bobwhite and 0.34 mm for mallards.

(C) A test is unacceptable if more than 10 percent of the adult control birds die during the test.

(3) **Analytical measurements—**(i) **Statistical analysis.** Experimental groups should be individually compared to the control group by analysis of variance. Other accepted statistical methods may be used as long as they are documented. In particular, regression analysis is highly desirable if the data and number of dose levels allow the use of this technique. Sample units are the individual pens within each treatment level or control. Analysis should include:

(A) Body weight of adults.

(B) Food consumption of adults.

(C) Percentage of hens laying eggs. This should always be determined when pens contain a single pair; if feasible, it should be determined when pens contain groups.

(D) Number of eggs laid per pen.

(E) Percent and number of cracked eggs.

(F) Percent viable embryos and number of eggs set.

(G) Percent live 18–day embryos of viable embryos.

(H) Percent and number hatching of viable embryos.

(I) Percent and number of hatchlings that are normal.

(J) Percent 14–day–old survivors of normal hatchlings.

(K) Number of 14–day–old survivors per hen.

(L) Body weights of 14–day–old survivors.

(M) Eggshell thickness.

(ii) **Test substance concentrations.** (A) Samples of treated diets should be analyzed to confirm proper dietary concentrations of the test substance. If samples cannot be analyzed immediately, they should be stored appropriately (e.g. frozen at a temperature of ·15 °C or lower) until analysis can be performed. Analyses should be conducted on all test substance concentrations at the beginning of the test period and again 10 to 12 weeks later. If not otherwise available, data should be generated to indicate whether or not the test substance degrades or volatilizes. If the test substance is known or found to be volatile or labile to the extent that 20 percent or more loss occurs within 1 week, then test substance diets should be prepared (freshly or from frozen concentrate) at a frequency that will prevent more than 20 percent loss of test substance.

(B) The assay method used to determine actual concentrations should be reported according to paragraph (f)(1)(vi) of this guideline.

(iii) **Analysis of basal diet.** A nutrient analysis of the basal diet should be included with the test report. For commercially prepared basal diets, the list of ingredients supplied by the manufacturer is normally sufficient if it is detailed. The composition of any vitamin or other supplements should also be reported.

(e) **Test conditions—**(1) **Test species—**(i) **Selection.** (A) The bobwhite, *Colinus virginianus* (L.), or the mallard, *Anas platyrhynchos* (L.), is the test species. Test birds should be pen-reared. They may be reared in the laboratory or purchased from commercial breeders. Rearing stock and/or tests birds should be obtained only from sources that have met the requirements for ‘‘U.S. Pullorum-Typhoid Clean’’ classification under paragraph (g)(1) of this guideline. Birds should be obtained only from sources whose colonies have known breeding histories. If possible, a history of rearing practices for test birds should be obtained and made available upon request. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and

exact age. Test birds should be phenotypically indistinguishable from wild stock. Conscientious breeders of such birds will periodically outbreed their flocks with genetically wild stock in order to maintain a genetic composition that approximates the heterogeneity of naturally occurring birds.

(B) All control and experimental birds used in a test should be from the same source and strain. If shipped, all birds should be examined following shipment for possible physical injury that may have occurred in transit. All birds should have a health observation period of at least 2 weeks prior to selection for treatment. Birds should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. A population of birds should not be used if more than 3 percent of either sex become debilitated during the health observation period. Birds should not have been selected in any way for resistance to toxic substances. Birds should not have been used in a previous test, either in a control or treatment group. Offspring of birds used in a treatment group in a previous test should not be used, but offspring of birds used as a control in a previous test are acceptable.

(C) Tests birds should be approaching their first breeding season and should be at least 7 months old. All test birds should be the same age within 1 month. The age of test birds should be reported.

(D) Bobwhite should be acclimated to test facilities and untreated basal diet for at least 2 weeks. Acclimation may be in the actual pens used in the test or in identical pens. The acclimation period may coincide with the health observation period. Birds should be randomly assigned to treatment and control pens. However, when birds in a pen are incompatible, they may be rearranged within a control or treatment group at any time prior to initiating treatment.

(E) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) **Diet**—(A) **Adult birds.** (*1*) A standard commercial game bird breeder ration, or its nutritional equivalent, should be used for diet preparation. This ration or basal diet should be used for both control and treatment birds and should be constant throughout the duration of the study. Antibiotics or other medication should not be used in the diet or water of breeding birds. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants. However, diets should be analyzed periodically for these substances and should be selected to be as free from contaminants as possible. A nutrient analysis (quantitative list of ingredients) of the diet should be included with the test report.

(*2*) The test substance should be mixed into the diet in a manner that will ensure even distribution of the test substance throughout the diet.

If possible, the test substance should be added to the diet without the use of a carrier or diluent. If a diluent is needed, the preferred diluent is distilled water; but water should not be used for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g. acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should comprise no more than 2 percent by weight of the treated diet, and an equivalent amount of diluent should be added to control diets.

(*3*) Diets may be mixed by commercial or mechanical food mixers. Other means are acceptable as long as they result in even distribution of the test substance throughout the diet. Screening of the basal diet before mixing is suggested to remove large particles. For many tests substances, it is recommended that diets be mixed under a hood. Frequently, the test substance is added to an aliquot of the basal diet to form a premix concentrate. The premix concentrate should be stored so as to maintain the chemical concentration. For final preparation of test diets, the premix is mixed with additional basal diet to form the proper concentrations. The frequency with which final treated diets are prepared will depend upon the stability and other characteristics of the test substance. Unless otherwise specified or determined by degradation or volatility studies, it is recommended that final diets be prepared weekly, either fresh or from a concentrate. For volatile or labile test substances, test diets should be mixed frequently enough so that the concentrations are not reduced from initial concentrations by more than 20 percent. Analysis of diets for test substance concentration is required as specified in paragraph (e)(1)(ii) of this guideline.

(*4*) Clean water should be available ad libitum. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed daily or more often.

(B) **Young birds.** Young birds produced during the test should be fed a commercial game bird starter ration, or its nutritional equivalent. No test substance should be added to the diets of young birds. No antibiotics or medication may be used in the diet. Bacitracin, or one of its forms, may be added to the drinking water of young birds, if necessary.

(2) **Facilities.** (i) Bobwhite should be housed in breeding pens or cages of adequate size conforming to good husbandry practices. Space requirements for bobwhite have not been well defined, but it is recommended that there be at least 5,000 cm^2 (approximately 2.3 ft^2) of floor space per bird. Space requirements for mallards have not been well defined, but it is recommended that there be at least 10,000 cm^2 (approximately 5.4 ft^2) of floor space per bird. Documentation that reproductive

parameters and health of birds are not adversely affected should be provided for cages much smaller than this area. The preferred construction materials are stainless steel, galvanized sheeting, and wire mesh. For enclosed cages, floors and external walls may be wire mesh; ceilings and common walls should be solid sheeting. Wire mesh for floors should be fine enough so as to not interfere with normal movement of the birds. Open-topped pens may be constructed of the same materials for the side walls with open tops and wire mesh or concrete floors. Concrete floors should be covered with litter such as straw, wood shavings, or sawdust. Other construction materials, except wood, are acceptable if they can be kept clean. Wood may be used as vertical framing posts for the support of wire mesh or for horizontal framing along the top of the pen. Wood should not be used for floors or lower sides of pens unless it has been coated with a nonadsorbent material such as perfluorocarbon plastic (e.g. Teflon), or unless the wood is replaced between tests.

(ii) Pens should be disassembled (if feasible) and should be cleaned thoroughly between tests. Steam cleaning of enclosed cages is recommended. Enclosed cages may be brushed thoroughly, as an alternative method. For open-topped pens, the sides and vertical supports should be thoroughly brushed. Any used floor litter should be discarded. The floor composition will dictate methods used to clean the floor. If litter is used on the floor, it should be fresh and clean when birds are placed in the pen. The use of detergents or bleach is acceptable, but other chemical disinfectants (such as quaternary ammonium compounds) should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended.

(iii) Pens should be kept indoors in order to better control lighting, temperature, humidity, and other factors. Outdoor pens may be used only during the normal breeding season. The photoperiod should be carefully controlled, preferably by automatic timers. A 15 to 30 min transition period is desirable. The photoperiod regime is described under test procedures under paragraph (d)(2)(v) of this guideline. Lights should emit a spectrum simulating that of daylight. The use of shorter wave-length ‘‘cool-white’’ fluorescent lights that do not emit the daylight spectrum should be avoided. Illumination intensity should be about 6 fc at the level of the birds.

(iv) Temperature and humidity should be controlled during the study. Recommended levels are 21 °C and 55 percent relative humidity. Temperature should be recorded at least weekly at the same time of day and should be reported. For tests conducted without temperature control, temperature minimums and maximums should be recorded daily. Continuous temperature monitoring is desirable. Temperature recordings should be made at a level of 2.5 to 4 cm above the floor of the cage. Recording

of approximate humidity levels is also desirable. Good ventilation should be maintained. Suggested ventilation rates are 4 changes per hour in winter and 15 changes per hour in summer.

(v) If facilities are being used for the first time, it may be desirable to allow birds to breed in the facility prior to testing in order to ensure that controls will have acceptable productivity according to the requirements given in paragraph (d)(2)(xi) and (xii) of this guideline.

(f) **Reporting.** (1) The test report should include the following information:

(i) Name of test, sponsor, test laboratory and location, principal investigator(s), and actual dates of beginning and end of test.

(ii) Name of species tested (including scientific name), age of birds (in months) at the beginning of the test, source of birds, and body weights for adult birds throughout the test.

(iii) Description of housing conditions, including type, size, and material of pen, temperature, humidity, photoperiod and lighting intensity, and any changes during the test.

(iv) Detailed description of the basal diet, including source, composition, diluents (if used), and supplements (if used). A nutrient analysis of the basal diet should be included.

(v) Detailed description of the test substance including its chemical name(s), source, lot number, composition (identity of major ingredients and impurities), and known physical and chemical properties pertinent to the test (e.g. solubility, volatility, degradation rate, etc.).

(vi) The number of concentrations used, nominal and measured concentrations of test substance in each level, assay method used to determine actual concentrations, storage conditions and stability of treated diets, number of birds per pen and number of replicate pens per concentration and for controls.

(vii) Acclimation procedures and methods of assigning birds to test pens, including method of randomization.

(viii) Frequency, duration, and methods of observation.

(ix) Description of any signs of intoxication or any other abnormal behavior, including time of onset, duration, severity (including death), and numbers affected (including accidental deaths or injuries), and any remissions.

(x) Food consumption per pen and any observations of repellancy or food palatability.

(xi) Method of marking all birds and eggs.

(xii) Details of necropsies.

(xiii) Egg and hatching data in summary and by pen per week in sufficient detail to allow an independent statistical analysis. Data should be presented for all of the parameters listed in paragraph (d)(3)(i) of this guideline. The number of eggs set should also be reported.

(xiv) Egg storage, incubation, and hatching temperatures, relative humidities, and turning frequencies.

(xv) Observations of health and weights of young at 14 days of age; food consumption of young—first and second week after hatching.

(xvi) Location of all raw data storage.

(xvii) Methods of statistical analysis, interpretation of results (including NOEC and any statistically significant effect levels), and sufficient data to verify calculation of statistical values.

(xviii) Results of residue analysis (if performed).

(xix) Test parameters are to be reported using the metric system.

(xx) Names of toxicants (if any) used as reference substances and method of preparation of test concentration.

(xxi) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(2) In addition, the following information should be available upon request:

(i) A general description of the support facilities.

(ii) A description of the quality control/quality assurance program, including the average quality level (AQL) for the program element performing the test, procedures used, and documentations that these levels have been achieved.

(iii) The names, qualifications, and experience of personnel working in the program element performing the test, including the study director, principal investigator, quality assurance officer, as well as other personnel involved in the study.

(iv) Standard operating procedures for all phases of the test and equipment involved in the test.

(v) Sources of all supplies and equipment involved in the test.

(vi) Diagram of the test layout.

(vii) Originals or exact copies of all raw data generated in performing the test.

(viii) A detailed description, with references, of all statistical methods.

(g) **References.** For additional background information on this test guideline the following references should be consulted:

(1) U.S. Department of Agriculture. National Poultry Improvement Plan, Report No. 2., in *Directory of Participants Handling Waterfowl, Exhibition Poultry, and Game Birds.* USDA, Science and Education Administration, Beltsville, MD 20705 (1979).

(2) [Reserved]